Bacterial Profile Associated With Appendicitis

Naher, H. S. and F. K. Ktab

College of Medicine, University of Babylon, Iraq

Abstract: The study included the detection of bacterial types in 110 excised appendix being taken from 110 patients having acute appendicitis who were referred to Al-Kufa Teaching Hospital, department of urology whose ages ranged from 4 to 60 years old. The patients were 69 (62.7%) males and 41(37.3%) females.

The clinical features of patients being observed by physicians were recorded. Those were right iliac fosse pain, generalized abdominal pain, fever, loss of appetite and nausea. White blood cells count, C-reactive protein and general urine analysis were also studied, in addition to abdominal ultrasonography and computer tomography (C.T.). The age group of both sexes being more susceptible for appendicitis ranged from 11 to 20 years old. The ratio of males to females' infections was 1.7:1. A total of 111 bacterial isolates were isolated from inflamed appendicitis of 110 patients with acute appendicitis. Positive bacterial cultures were detected in 90 (81.8%) patients while 20 (18.2%) patients showed no growth. The aerobic bacteria accounted for 87 (78.4%) isolates whereas anaerobic were only 24(21.6%) isolates. Gram-negative bacteria were presented in 107 (96.4%) while gram-positive bacteria were accounted for 4 (3.6%).

Escherichia coli was the predominant pathogens, since it accounted for 36 (32.4%) of all isolates followed by *Bacteroides* spp. 21 (18.9%), *Klebsiella pneumoniae* 18 (16.2 %), *Pseudomonas aeruginosa* 11 (9.9%), *Citrobacter freundii* 7 (6.3%), *Salmonella typhi* 5 (4.5%), *Proteus mirabilis* 5 (4.5%), *Enterobacter aerogenesa* 4 (3.6%), *Peptodtreptococcus* 2 (1.8%), *Staphylococcus aureus* 1 (0.9%) and *Clostridium perfringns* 1 (0.9%).

Mixed cultures were detected in 21 cases*****, in which more than one organism were detected. Most of mixed bacterial isolates were aerobic with anaerobic bacteria 13 (61.9%) in which *Escherichia coli* was the common, since it accounted for 15 (71.4%).

***** Full presentation for this data is not shown in this paper.

Introduction: The appendix is a blind-ending structure that arises from cecum. It is usually referred to as a functionless organ but may play a role in immunity (Zuercher, *et al*., 2002).

Acute appendicitis is one of the most frequent condition that leads to emergent abdominal surgery (WHO, 2004). Appendicitis is due to a closed-loop obstruction of the appendix which is usually due to either lymphoid hyperplasia within the appendix or impacted fecal matter which is referred to as a fecalith (AL-Joubori, 1994). Obstruction leads to bacterial overgrowth which leads to an increase in intra-luminal pressure which obstructs the blood flow and leads to congestion and ischemia in the appendix allowing the bacterial translocation and infection resulting in the cause inflammation of appendix (Anderson and Bergdahi, 1978).

As the infection progresses the inflammation advances to gangrenous. This leads to the appendix, consequently inflammatory fluid and bacterial contents spill and release in to abdominal cavity (Shelton, *et. al*., 2003).

A variety of bacterial species have been reported to play a major role in appendicitis. Both, aerobic and anaerobic, gram-negative and gram-positive bacteria have been reported to be implicated in appendicitis such as *Bacteroids fragilis* (Gilbert, *et., al*., 2002), Beta-hemolytic streptococci (Jacobsen, *et al.*, 1987), *Yersinia enterocolitica* (Okoro, 1988) *Eschrichia coli* (Guasco, *et al*., 1991) *Klebsiella* spp. (Leigh, *et al*., 1974) *Citrobacter frundii* (Parkhomenko, 1998).

Most studies focused on the diagnosis of appendicitis. Othe dtudies dealt with parasitic infection but very rare studies have been done regarding the bacterial infection of appendicitis. Therefore this study was suggested and designed to fulfill the following goals:

Detection the bacterial profile associated with appendicitis.

Determination the common bacterial causes of appendicitis.

Determination the risk factor associates with appendicitis.

Materials and methods:

The subjects: A total of 110 patients (69 males and 41 females) who were referred to AL-Kufa Teaching Hospital in Najaf and diagnosed by physicians as acute appendicitis. Their ages ranged from 4 to 60 years. All patients were under the follow up laboratory investigation. General urine examination (GUE), White blood cells count (WBCs) and C-reactive protein were tested for them. Ultra-sonography and computerized tomography (C.T.) scanning being suggested by physicians were also considered.

Materials: The necessary instruments, chemicals, and biological substances were properly used in this study. The traditional culture media were used for routine diagnosis bacterial isolates. Peptone supplemented with 5% glucose was used as recommended by Abid AL-Sada (1999) to transfer the specimens to the laboratory for bacteriological analysis and inflame heated spatula

(cauterization) method was used for sterilization the outer surfaces of excised appendix Abid AL-Sada, 1991).The following diagnostic systems were used to confirm the diagnosis of bacterial isolates: (all of these systems were supplied by Analytab Products Co.)

API 20E system for identification of gram-negative bacilli.

API 20S system for identification of streptococci.

API *Staph*. for identification of staphylococci.

API 20A system for identification of anaerobes

Statistical analysis: The data were statically analyzed using Chi-square (X^2) test and Z-test at 1% level (Daniel, 1978).

Results and discussion:

The clinical features related to appendicitis are shown in table-1. Those symptoms were recorded under the advice of physicians. Most of the patients (84) included in this study were complaining from abdominal pain, either in right lower quadrant 70 patients (63.6%) or generalized pain 14 patients (12.7%), while most patients were febrile but low- grade fever characterized by flushness of the cheeks has been seen in 35 patients (31.8%). Nausea and (sever) constipation have been observed in 31 patients(28.1%) and 16 patients (14.5%) respectively. Diarrhea and vomiting were seen in 11(10.0%) and 6 patients (5.5%) respectively. The results were in accordance with the results obtained by Kosloske (2004) who stated that the right iliac fosse is the common features of appendicitis. Katzung (2003) stated that, the classic description of appendicitis is vague peri-umbilical pain followed by nausea, vomiting and anorexia. If the pain initially located in the right lower quadrant, sever constipation should be considered (Katzung, 2003). Likewise, significant diarrhea is atypical in appendicitis. Patients with appendicitis relate symptoms of frequent, small-volume, soft stools and usually not true diarrhea. According to Katzung, (2003), vomiting and fever are more frequent in patients with appendicitis than in patients with other causes of abdominal pain.

Table-1: distribution of patients according to clinical features (n=110)

Clinical features	**N(%)**
Right iliac fosse pain	70(63.6)
fever	35(31.8)
Nausea	31(28.1)
Constipation	16(14.5)
Generalized pain	14(12.7)
Diarrhea	11(10.0)
Vomiting	6(5.5)
Anorexia	6(5.5)

According to the age, appendicitis occurs in all age groups, but the results revealed that, significant differences upon age factor (Cal. X2 = 18.8, tab. X =7.81, P< 0.05) since the peak of incidences observed in the age group of 11 to 20 years old as shown in table 2, that the frequency of infection accounted for 57(51.8%) patients , 39(35.4%) of them were males and 18(16.3%) females (the male-to-females ratio is approximately 2.1:1 among this group only). The results indicated that the appendicitis incidences decreased with the advancement of age, since the lowest rate 2(1.8%) was seen within the age group of 51 to 60 tears old. Such results can ascribed to the nature of physiological and anatomical reasons of appendix tissues (Jones, 2002), since lymphoid tissue is the most susceptible for infection gradually increases up to 20 years of age and then begins to decrease with advancement of the age up to 60 years old when it is totally disappear. In contrast appendicitis has been reported to be very rare in neonates (Katzung, 2003).

Back to table 2, the results indicated that males 69(62.7%) are significantly (Cal. Z= 2.126, tab. Z= 0.984, P< 0.09) more susceptible than females 41(37.3%) with a total ratio of 1.7:1. Similar results have been reported by Addiss, *et. al*, (1990), AL-Fahad (2003) and Katzung (2003) who stated that the male-to-female ratio is approximately 2:1. That because the diagnosis of acute appendicitis in males is more reliable compared with females, that misdiagnosis in females may result due to other diseases such as gynecologic diseases.

Table-2: Distribution of patients according to age and sex (n=110)

Age/year	**No. patients (%)**	**Males (%)**	**Females (%)**
4-10	3(2.7)	1(0.9)	2(1.8)
11-20	57(51.8)	39(35.4)	18(16.3)
21-30	33(30)	20(18.1)	13(11.8)
31-40	9(8.1)	5(4.5)	4(3.6)
41-50	6(5.4)	3(2.7)	3(2.3)
51-60	2(1.8)	1(0.9)	1(0.9)
Total	110	69(62.7)	41(37.3)

Table 3 below, shows that 90 specimens (81.8%) out of 110 specimens (appendix swabs) yielded positive results for bacterial culture, while 20 specimens (18.2%) showed no growth. Among the positive growth, 65(59.1%) were males origin while 25(22.7%) were from females.

Table-3: Culturing of swabs from inflamed appendix.

Cultural- results	**n (%)**	**Males (%)**	**Females (%)**
Positive	90(81.8)	65(59.1)	25(22.7)
Negative	20(18.2)	4(3.6)	16(14.6)
Total	110	69(62.7)	41(37.3)

A total of eleven bacterial genera were detected through this study, three genera belong to gram-positive bacteria and eight genera belong to gram-negative as shown in table 4. Gram-negative isolates were the common cause of appendicitis since they accounted for 107(96.4%) versus only 4 isolates (3.6%) gram- positive in addition to 21 cases (18.9%) which revealed mixed growth (more than single organism).

Table-4: Frequency of bacterial isolates detected in appendicitis.

Type of bacterial isolate	**N (%)**
Gram-positive bacteria	**107(96.4)**
Escherchia coli	36(32.4)
Bacteriodes spp.	21(18.9)
Klebsiella pneumoniae	18(16.2)
Pseudomonas aeruginosa	11(9.9)
Citrobacter frudii	7(6.3)
Salmonella typhi	5(4.5)
Proteus mirabilis	5(4.5)
Enterobacter aerogenes	4(3.6)
Gram-positive bacteria	**4(3.6)**
Peptostreptococcus sp.	2(1.8)
Closridium perfriges	1(0.9)
Staphylococcus aureus	1(0.9)
Mixed growth*	21(18.9)
Total	131

The most frequent pathogen was *E. coli* which accounted for 36 isolates (32.4%) followed by *Bacteroides* spp.; 21(18.9%), *Klebsiella pneumoniae*; 18(16.2%) and so on for the other bacteria presented in table 4 above. The results were in accordance with other results being reported by Elhag, *et. al*., (1986) and Baste *et. al*. (1999). The results were accepted and suspected, since *E. coli* is the common organism in intestine, fast proliferates and quickly

***** Full presentation for this data is not shown in this paper.

adheres to the tissue surfaces (Baron, *et. al*., (1992). The adhesion of microorganism to the epithelial cells is the first step of infection followed by the invasion step. *E. coli* has other virulence factors represented by haste-cell-surface-modifying factor, toxins, hemolysin and cytotoxin necrotizing factor type 1 – CNF1. (Beachy, *et.al*., 1981).

Bacteroides has been also reported to possess several factors capable to develop intra-abdominal infections by three ways; stimulation of abscess formation, reduction of phagocyte by polymorph-nuclear leukocytes (PMNL), because of the capsule of *Bacteriodes* and the ability to produce Beta-lactamase which inactivates the antibiotics (Kenneth, *et. al*., (2003). Other gram-negative bacteria represented by *K. pneumon*, *C. frundii, S. typhi, P. mirabilis and E. aerogeneswere* also detected to implicate in appendicitis although in low frequencies compared with other members of gram-negative bacteria (table- 4). However, the implication of these bacteria in appendicitis is suspected, since they belong to enteric group (*Enterobacteriaceae*) and frequently present in intestine and all have virulence factors enabling them to adhesion, invasion and causing infections (Abbott, 1999). The explanation for detection of *Pseudomonas aeuginisa* in appendicitis can be attributed to the ability of this organism to adhere and strongly colonizes the epithelial tissue probably by pili and the algenet (a slime later) surrounding the cells of this bacterium (Zhu, *et. al*., 2004). Moreover, *P. aeroginosa* possesses active in producing enzymes and toxins which enable the bacteria to cause diseases.

In this study, some of gram-positive bacteria represented by *Staphylicoccus aureus, Peptostreptococcus* sp., and *Clostridium perfringes* were also isolated from appendicitis cases, even in low frequencies in relative with that of gram-negative (table-4).

Members of gram-positive appendicitis are rarely reported at the present time. This may due to adhesive and colonizer factors being less among gram-positive bacteria compared with that of gram-negative. Moreover, qualitatively, gram-positive infections are most serious and the detection of *Clostridium welchii* during this study can be the announcement that the obligate anaerobic necrotizer gas gangrene causative agent, *Cl. welchii* can implicate in appendicitis.

References:

1- Zuercher, A.W., S.E.Coffin, M.C. Thurnheer, P. Fundowa and J.J. Cebra (2oo2). Nasal associated lymphoid tissue a mucosal inductive site for virus. Specific humoral and cellular immune responses. J. Immuno.**168:** 796-803.
2- WHO (2004), Map of graphic: Countries by Mortality; Acute appendicitis.
3- AL-Joubori, A.K. (1994). Acute appendicitis: Clinical, histopathological and bacteriological study. Dipl. thesis. College of medicine, University of Baghdad.

4- Anderson, A. and I. Berghahi (1978). Acute appendicitis in patients over 60. Ann. Surg. **44:** 445-447.
5- Shelton, T., R. lefering and R.W. Schwartz (2003). Acute appendicitis. Current diagnosis and treatment. **60:** 502-505.
6- Gilbert, D.N., R.C. Moellering and M.A. Sande (2002). The Sanford guide to antimicrobial therapy. Hyde Park. Vermont: Antimicrob. Ther. Inc. pp.314
7- Jacobsen, J., J.C. Andersen and I.B. Klausen (1987). Beta haemolytic streptococci in acute appendicitis. Acta Chir. Scand. **154:** 30.
8- Okoro, I. (1988). The role of Yersina enterocolitica in appendicitis in Zaria. East Afr. Med. J. **65:** 625.
9- Guasco, C., F. Roncheto, P. Milani, E. Stocchini and P.G. Pistono (1991). Bacteriology of abdominal pus in 43 cases of acute appendicitis and appendicle abscess at the Ivra-Castellamonate Hospital: isolation of aerobic and anaerobic bacteria and drug sensitivity. J. Bacteriol. Virol-Immunol. **84:** 77.
10- Leigh, D.A., K. Simmon and E. Norman (1974). Bacterial flora of appendix fosse in appendicitis and post operative wound infection. J. Clin. Path. **27:** 997.
11- Parkhomenko, I.U., L.S. Lozovskaia and V.A. Giunko (1991).The immunomorphological characteristics of the appendix in a viral-bacterial lesion in children with appendicitis. Arkh. Patro. **53:** 33.
12- Daniel, W.W. (1978). Biostatic. A foundation for analysis in the health science. Ed. John Wiley and Sons. New york.
13- Abid Al-Sada, H. G. (1999). Bacteriological study on excised tonsils from patients in Babylon province. M.Sc. thesis, College of science, University of Babylon.
14- Kosloske, A. (2004). The diagnosis of appendicitis in children: outcomes of a strategy based on pediatric surgical evaluation. Ped. J. **62:** 68.
15- Katzung, B.G.(2003). Basic and clinical pharmacology. 8th. ed. McGraw-Hill. Pp37.
16- Jones, P.F.(2002). Suspected acute appendicitis trends in management over the past 30 years Br. J. Surge.**88:** 1570-77.
17- Addiss, D.G., N. Shaffer, B.S. Fowler and R.V. Tauxe (1990). Epidemiology of appendicitis and appendectomy in U.S. Am. J. Epid. **132:** 910-25.
18- AL-Fahad, N.K.(2003). Application of the modified score in the diagnosis of acute appendicitis. Ph. Thesis, College of medicine, Univ. Kufa.
19- Elhage, R.M., Alwan, M.S., A.L. Adnahi and R.A. Sheriff (1986). *Bacteroides fragilis* is a silent pathogen in acute appendicitis. Med. Micrbiol. **21:** 245.
18- Baste, M., N.E. Morton, J.I. Mulvihill, Z. Radovanvic, I. Radojicic and D. Marinlcovic (1999). Inheritance of acute appendicitis: familial aggregation and evidence of polygenic transmission. Am. J. Human Gen. **46:** 377-82.
19- Baron, E.J., L.R. Peterson and S.M. Finegold (1994). Diagnostic microbiology, 9th. ed. Bailey and Scotts', C.V. Mosby Com.

20- Beach, E.H.(1981). Bacterial adherence: adhesion receptor interaction mediating the attachment of bacteria to mucosal surface. J. Infect. Dis. **143:** 325-345.
21- Kenneth, E.A., A. Deborah, O. Megan and Vocharles (2003). Bacteremia due to *Bacteroides fragils* group. Ant. Ag. Chema. **47:** 148-53.
22- Abbott, S.(1999) *Klebsiella*, *Enterobacter*, *Citrobacter* and *Serratia*: Manual of clinical microbiology.7th. ed. Am. Soc. Micr. pp 92.
23- Zhu, L.M., D. Cai, Y. Lu, W.H. Chen, W. F. Wang and Y.L. Zhang (2004). The study of pro-nucleating activity of bacteria identified in cholesterol gall stones in model bile system. Waike Zazhi. **42:** 1501-5